Antibiotics Feeding their Impacts Warnings Threats to Human and Animal Health and Some Remedies

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Let food be thy medicine and medicine by thy food"

It was proclaimed by some 2500 years ago by Hippocrates. An Antibiotic is produced by a microorganism that inhibits or kills other microorganisms. The word 'antimicrobials' is often used to encompass any substance of natural or of synthetic origin (chemotherapeuticals).

A century ago first antimicrobial (Salvarsan) was used for treating syphilis in 1910. Use of antimicrobials was expanded in the 1930's with the use of sulphonamides (first chemotherapeutical) in human medicines. Penicillin though discovered in 1929 by Alexander Fleming was introduced for therapeutic use in 1941 for curing various bacterial diseases. Antimicrobials are the single most important discovery in the history of medicine being considered as miracle drugs because of saving millions of human and animal lives. Table 1 depicts the history of treatment of ailments.

Table 1 Use of medicines for treating ailments

Year	Prevailing Medical advice
2000 BC	Here eat this root
1200 AD	This root is heathen , say this prayer
1500 AD	Prayer are superstitious, drink this potion
1900 AD	This potion is snake oil, Swallo this pill
1950 AD	This pill is ineffective, take this antibiotic
2000 AD	This antibiotic is synthetic, eat this root.

Antimicrobials As Growth Promoters (AGP's)

Growth promoting properties of antimicrobials in poultry was first reported by Moore et al. (1946) and in pigs by Jukes et al (1950). Feeding of sub-therapeutic doses of antimicrobials to the farm animals was readily adopted. Various antimicrobials used in animal/ poultry production are given in the table 2.

Table2. Antimicrobial agents approved for use in broiler production

Coccidiostats (antiprotozoal drugs)

Sulfonamides	Ionophores	Others
Sulfachloropyrazine Sulfamethazine Sulfadimethoxine Sulfamyxin Sulfanitran Sulfaquinoxaline	Lasalocid Maduramycin Monensin Narasin Salinomycin	Amprolium Arsanilate (arsenical) Buquinolate Clopindol Dequinate Nequinate Robenidine Zoalene

Antibiotics and arsenicals

Aminocyclitols (spectinomycin); Aminoglycosides (streptomycin, neomycin, gentamycin); Arsenicals (roxarsone, arsanilic acid); Bacitracin* Decapeptides (bacitracin); Bambermycin; B-Lactams (penicillins); Carbadox;; Erythromycin*; Fluoroquinolones (enrofloxacin, sarafloxacin); Laidomycin; Lasalocid; Lincomycin*; Lincosamides (lincomycin); Macrolides (erythromycin, tylosin); Monensin; Novobiocin Oleandomycin; Roxarsone; Streptogramins (virginiamycin);

Tiamulin; Tetracyclines (chlortetracycline, oxytetracycline, tetracycline);
* Identical to human-drugs

[a]Adapted from NRC, 1999 and USDA, 2001. [b]Labeled as growth promoter [c]The most commonly used arsenical compound in poultry feed. SOURCE: Hancock TC, Miller CV, Denver JM, Riedel GF. Fate and transport of arsenical fee amendments in Chesapeake Bay watersheds. Society of Environmental Toxicology and Chemistry 21st Annual Meeting; 2000 Nov 12–16; Nashville, TN.

Use of antibiotics as growth promoter is defined as antibiotics provided to healthy animals at concentrations below 200 grams per ton of feed for more than 14 days (FDA, 2000). While therapeutic doses are very high. Small doses result in conditions which are particularly conducive to selection for resistance in bacteria. Microbial resistance to antibiotics is on the rise, and is even becoming something of a crisis in some countries' health care. For bacteria to develop resistance to antibiotics, they must first receive exposure to them. Overuse of prescribed drugs in human and veterinary medicine, as well as their use as growth promoters in animals has all been blamed. The recommended inclusion levels in diets poultry and pig were 4g per ton for narrow-spectrum and 10 g per ton of the broad- spectrum antibiotics in 1950's, but increased 10 to 20 fold since then. Worldwide legislation to control use has eventually fuelled the downfall of antibiotic use. Consumers may still demand antibiotic treatment from doctors where it is ineffective, but they are no longer so keen to eat meat, which has been raised with their help. As per WTO treaty, the sanitary and phyto-sanitary measures are to be used to an extent required to protect animals and plants that too with in scientific principles. The chance for residual effects can be ignored though the farmers are advised to withdraw antibiotic feed additives seven to ten days prior to sacrifice the meat birds. Moreover, there is transition of the society from cheap food to "safe" food. Animal welfare activists demand for natural products. The net result has been world over rejection on antibiotics (Mandal and Elangovan 2004, Sluis 2005, Nollet, 2005, Wakeman, 2005). The use of ionophore antibiotics (monensin, lasolacid, etc.) has been extensive in certain countries for fattening of ruminant meat animals. The use of antibiotics as feed additives has been banned/efforts are being made to ban in many countries, but the experience has not been satisfactory, as the disease incidence has been increased resulting much more use of therapeutic antibiotics. However, the antibiotics are classified as feed antibiotics and therapeutic antibiotics to curb the use of therapeutic antibiotics at sub-therapeutic levels for animal production. The World Health Organization, the American Medical Association, and the American Public Health Association have urged a ban on growth promoting antibiotics (GPAs), arguing that their use leads to increased antibiotic-resistant infections in humans. In contrast, commercial interests have argued that their removal will have a significant impact on the cost of production and is unlikely to affect the risk to humans from antibiotic-resistant infections (Smith et al. 2002, Casewell et al. 2003). Studies have shown that the use of growth promoting antibiotics contributes to contamination of flocks and food products by antibiotic resistant pathogens, including *Campylobacter, Salmonella, Enterococcus* and *Escherichia coli* and thereby to increased risks of human infections by these and other resistant pathogens (Feinman, 1979, Cohen and Tauxe, 1986). The European Union, in 1999, banned the use of most antibiotics for growth promotion to preserve the effectiveness of important human drugs (Casewell et al. 2003). The U.S. has not adopted this broad policy, but in 2004 the U.S. Food and Drug Administration banned enrofloxacin (a fluoroquinolone applied for therapeutic uses, not growth promotion) in food animals on the grounds that its use contributed to fluoroquinolone resistance in human pathogens. In recent years, concerns about the use of

antimicrobial products in food-producing animals have focused on human food safety because foods of animal origin are identified as vehicles of foodborne disease in humans. As a result of treating animals with antibiotics, foodborne bacteria may also be resistant to the antibiotics used to treat human disease.

Benefits of AGP's

The overall outcome of use of AGP's is the availability of more nutrients for growth and production. The improvement of growth rate and feed conversion ratio (feed:gain) has been 16% & 9% in piglets, 9% and 5.5% in growing pigs, 3-10% and 3-5% in broiler chickens,2% and 1% in layers, 7-10% and 4-5% in veal calves (Avcare 2003).

Antimicrobials for therapeutic purposes are available on prescription however as growth promoters these are freely accessible and sold over the counter

Some effects of uses of AGP's on broad issues are depicted in table 3

Table 3. Effects of antimicrobial growth promoters on some broad issues of animal production.

Sr. No.	Broad issue	Positive effect	Negative effect
1	Health	Control of certain diseases (primarily enteric) to some extent	1. Development of antimicrobial resistance 2.Masks sub-clinical disease and infection 3.Limits incentives for hygienic improvements
2.	Welfare	Alleviates and dampens disease signs	1.Camouflages stress associated with sub-clinical disease 2. Allows higher stocking rates
3	Husbandry	Increased 1.Production & 2. Productivity	Stimulates, increase and intensify animal production
4	Feed	None	1.Camouflages bad feed quality 2. Hampers improvements in feed formulation 3.Development of alternatives
5	Production system	Lower labour demand due to more intense production methods	Hampers the development of animal-friendly production systems
6.	Environ ment	Better utilisation of feed; less manure	Increases the environmental pool of antibiotic resistance genes; antibiotic residues
7.	Human health	None	1. Transfer of resistance to humans 2. Increased societal costs for health care 3. Shortens economic life of medical antimicrobials 4. Occupational hazards through exposure to aerosol and dust contaminated with antimicrobials

Mechanism of action of AGP's:

Exact mechanism as to how AGP's promote growth is not entirely clear. It is widely assumed that AGP's act mainly through their effect on intestinal flora. With less than 10 % of intestinal microflora identified, there has been little chance of fully understanding AGP's mode of action. It is postulated that AGP's allow the animal to express their natural potential for growth, which is achieved through their direct influence on bacteria in the gut (Bedford 2005). AGP's are beneficial to the livestock as these help in reducing the total number of intestinal microorganisms. Secondly these may also create a more favourable balance between beneficial and non-beneficial ones. These are directly

responsible in depressing the microbial growth in the gastro-intestinal tract which is beneficial because of following reasons:

- Inhibition of sub clinical infections,
- Reduced gut motility
- Reduced mucin secretion
- Reduced toxin (eg. ammonia and biogenic amine from protein formulation) production
- Bile salts modification
- Thinning intestinal wall
- Increase in digestive enzyme output
- Improve the digestibility
- Increase the uptake of nutrients along the alimentary canal
- Reduce the opportunity for harmful bacteria to establish in the gut.
- Activation of the intestinal immune system
- Reduction in microbial use of nutrients thus sparing the nutrients for the host,

The ultimate impacts of use of antimicrobials as growth promoters in farm animal production system are as under:

1. Increased growth rate.
2. Better feed conversion
3. Improved egg production in laying hens
4. Increased litter size in sows
5. Un physiological early weaning of piglets
6. Increased milk yield in dairy cows.
7. Economize the animal production systems.
8. Allow high stocking rates which raise questions on animal ethics and welfare.
9. Provides some protection against certain diseases

Development of resistance

Antibiotics as routine feed additives are used at low concentrations, which appear to prevent some diseases. Over use of antimicrobials may diminish their effectiveness and the strains of resistant bacteria would arise. Of the 1,415 microorganisms known to cause diseases in humans, 60% are ZOONOTIC. Resistant genes are flowing freely between animal and human bacteria through the food chain, which makes the situation more alarming. Of great concern was the possibility that resistance generated on the farm could lead to a loss of effectiveness of key antibiotics in human medicine.

Microorganisms exist on the classical concept "*SURVIVAL OF THE FITTEST.*" Microbes produce certain compounds to kill other microorganisms for their own survival and propagation. In our language these compounds are called the Antimicrobials.

Antibiotic resistance means the ability of bacteria to loose their susceptibility to specific antibiotic drugs. Different mechanisms exist in bacteria that make them resistant to a specific or antibiotics of a common chemical group. Antibiotic resistance is conferred by variations in the genetic makeup of bacteria. The genes that confer antibiotic resistance are carried on the bacterial chromosome or on separate plasmids that can be transferred between different bacteria. Thus, one bacterium can transfer antibiotic resistance to another bacterium. In addition, genes that confer resistance to antibiotics tend to group together such that resistance to multiple antibiotics is transferred by a single plasmid. These properties of antibiotic resistance genes complicate the control of antibiotic resistance and contribute to the concern over the use of antibiotics in livestock and public health (Callan, 2003). Antibiotics can promote the establishment of an antibiotic resistant population of bacteria by killing the bacteria that are susceptible to the antibiotic but

leaving behind the bacteria that are resistant. Antibiotics do not induce the genetic changes that confer resistance, rather, they select for the bacteria that already have the genetic changes responsible for resistance. Indiscriminant or inappropriate use of antibiotics can promote the selection of antibiotic resistant bacteria. It is extremely important that antibiotics are used appropriately and according to the manufacturer's recommendations unless otherwise specified by veterinarian.

Warnings to the use of AGP's

Alexander Fleming warned with the statement that '**Microbes are educated to resist penicillin'**. By the early 1950s the problem of antimicrobial resistance was well acknowledged. Bacteria when exposed to antimicrobials develop strategies for their survival. One such strategy is development of resistance against the antimicrobial. Table 4 depicts some of the warnings and actions for the use of antimicrobials.

Table 4. Antimicrobials, Warnings and Actions

Sr. No	Year	Warnings / action
1	1945	Alexander Flemming warns against misuse of penicillin as 'microbes are educated to resist'
2.	1950s	Antibiotic resistance widely recognised — vertical transmission.
3.	1950	Tuberculosis bacteria resistant to Streptomycin.
4.	1953	Certain strains of dysentery bacillus was found resistant to Chloromphenicol, Tetracycline, Streptomycin, and Sulphanilamides
5	1958-1959	Tetracycline resistant to poultry.
6	1960s	Horizontal transmission recognized.
7	1969	Swann Committee recommends severe restrictions on antimicrobials supplementations in animal feeds.
8	1970s	Swann committee recommendations implemented in the UK and EU
9	1975	Swann committee recommendations relaxed: tolysin and spiramycin still permitted as growth promoters; vancomycin comes into use.
10	1977	Swedish Agriculture Board considers potential risk of antibiotic resistance, but concludes it is negligible
11	1984	Swedish farmers ask for government ban on antimicrobials in animal feed because of health and consumer concerns.
12	1985	Swedish ban on grounds of antibiotic resistance in animals and it's 'uncertain' long-term effects
13	1995	Avoparcin and Vancomycin resistant Enterococci in pigs and poultry.
14	1995	Norway banned Avoparcin
15	1996	German government banned Avoparcin
16	1997	EU banned Avoparcin
17	1997	Swedish report concludes that risk of antibiotic resistance in humans is 'far from negligible'
18	1997	WHO scientific meeting concludes that it is 'essential to replace growth promoting antimicrobials'
19	1998	Danish government banned Virginiamycin due to Streptococcus resistance.
20	1998	EU bans four antimcirobials in animal feed as 'precautionary' measure.
21	1999	EU Scientific Steering Committee recommends phase-out of antimicrobials that may be used in human/animal therapy

22	1999	Pharmaceutical industry opposes EU bans and takes EU to the European Court; judgement expected end 2001
23	2000	WHO recommends ban on antimicrobials as growth promoters if used in human therapy and in absence of risk-based evaluation.
24	2004	US FDA banned the non therapeutic use of Enrofloxacin for growth promotion in food animals.
25	2006	EU completely banned the use of AGP's
26	2008	US congress passed a legislation regarding AGP's

Originally it was thought that only one type of resistance vertical transmission i.e. Resistance through mutation in existing genes that is the resistant trait would be confined to the mutant clone and spread of resistance is confined to that clone only. Later on another type of resistance called Horizontal transmission i.e. The resistance could also be developed through the uptake of existing genes. In this case, the resistance trait through mobile genetic elements can also spread to other bacterial clones, to other bacterial species and even to other genera.

Some of the infections which could normally be treated by specific antibiotics have turned out to be untreatable. For example *methicillin* was introduced in 1960 for the treatment of *Staphylococcus aureus* infection and with in a few years, *methicillin*-resistant *Staphylococcus aureus* (MRSA) strains were reported. Then in 1980, *fluoroquinolones* were introduced for treatment of MRSA, but a majority of *Staphylococcus* strains became resistant to *fluoroquinolones* with in a year. Even in India, there was report published in a news paper (The Telegraph, Kolkata, Monday, 11 December, 2006) that a bacteria displaying resistance to virtually all antibiotics known to humans have surfaced in the country, sending ripples of alarm among medical researchers who have called for nationwide surveillance and stepped up efforts to stem its emergence. Scientists at Banaras Hindu University Institute of Medical Sciences in Varanasi detected the "super bug" among strains of *Staphylococcus aureus* - bacteria which can cause life-threatening infections such as pneumonia and septicemia. Two strains of *Staphylococcus aureus* were found resistant to *Vancomycin*, the drug of last resort in the arsenal of conventional antibiotics.

The Swann Committee: United Kingdom government in 1968 established a committee under the chairmanship of Swann to examine transferable antimicrobial resistance and the consequences for human and animal health arising from the use of antimicrobials for growth promotion and in veterinary medicine. The principal recommendations were as follows

- Permission to supply and use an antibiotic without prescription for adding to animal feed should be restricted to the antibiotics, which are of economic value in livestock production.
- Have little or no application as therapeutic agents in man or animal;
- Will not impair the efficacy of a prescribed therapeutic antibiotic or antibiotics through the development of resistant strains of organisms.'
- Specific drugs recommendations i.e. 'Tylosin should not be available without prescription for use as a 'feed' antibiotic.'

In 1975 EU accepted the macrolides (Tylosin and Spiramycin) as growth promoters. This has probably been one of the major reasons for the widespread macrolide resistance in Enterococci and Campylobacter from pigs. Use of Avoparcin was extended to other species, such as adult cattle. One of the scientific arguments put forward to support the

use of antimicrobials for growth promotion was that the low dose presents a special case in selecting for resistance. However, the recent bans on Avoparcin, Virginiamycin and tylosin followed the publication of studies demonstrating that above view was wrong.

The Swedish ban: Swedish farmers were growing increasingly sceptical towards feed antimicrobials as the continued use of antimicrobials might harm consumer confidence.
Ministry of Agriculture proposed a new Feeding stuffs Act (Government Bill 1984/85) that the use of antimicrobials in feed should be restricted to treatment, prevention or cure of diseases i.e. their use for growth promotion should not be allowed due to i) risk of increased resistance ii) risk of cross-resistance to other substances and iii) the risk of increased susceptibility of animals to salmonella and other enteric pathogens.
In support of the Swedish view the Ministries of Agriculture of EU appointed a commission, which concluded that 'the risk for increased resistance associated with the general use of antibiotic growth promoters is far from negligible and the potential consequences are serious for both animal and human health' (SOU, 1997).
The ban of Avoparcin: In March 1995 on the availability of first report on the occurrence of avoparcin- and vancomycin-resistant enterococci in pigs and poultry (Klare *et al.,* 1995; Bates *et al.*, 1994; Aarestrup, 1995), the Danish farmers' organisations agreed with the feeding industry for a voluntary cessation of use of avoparcin in animal feed to reduce the spread of antimicrobial resistance. Danish government on 20 May 1995 imposed a ban with scientific background of

- Of cross-resistance between avoparcin and vancomycin;
- The resistance is transferable;
- use of avoparcin as a growth promoter selects for vancomycin-resistant enterococci
- Vancomycin-resistant enterococci can be transferred to humans via the food chain (DVL, 1995).

Scientific Committee on Animal Nutrition: In May 1996 the EU Scientific Committee on Animal Nutrition (SCAN) concluded that further evidence was required to establish a risk to human health, animal health or the environment caused by avoparcin and the ban came into force on 1 April 1997.
Danish ban of virginiamycin: On 16 January 1998, the Danish government banned all use of virginiamycin as a growth promoter in Denmark, due to a risk of selection of streptogramin-resistant enterococci in pigs and poultry (Aarestrup *et al.*, 1998).
European Union bans: EU Conference on 'The Microbial Threat' held in Copenhagen, Denmark, in 1998, stated: 'Most of those at the conference considered the use of antimicrobials for growth promotion was not justified and that it was essential to have a systematic approach towards replacing growth promoting antimicrobials with safer non-antimicrobial alternatives including better farming practice.' (The Copenhagen Recommendations, 1998)
:In July 1999, the agriculture ministers of the EU Member States as 'a precautionary measure to minimise the risk of development of resistant bacteria and to preserve the efficacy of certain antibiotics used in human medicine' imposed ban on the use of virginiamycin, bacitracin zinc, tylosin phosphate and spiramycin.The latest example of an antimicrobial growth promoter from the EU list of approved products showing cross-resistance with a potential human drug (everninomycin) is avilamycin (Aarestrup, 1998).
Scientific Steering Committee: In 1999 the Scientific Steering Committee (SSC) of EU concluded in its report that 'action needs to be taken promptly to reduce the overall use of antimicrobials in a balanced way in all areas: human medicine, veterinary medicine, animal production and plant protection' (SSC, 1999).

In relation to antibiotics for animal growth the SSC recommends that ‘the use of agents from classes which are or may be used in human or veterinary medicine should be phased out as soon as possible and ultimately abolished’.

World Health Organisation: In 1997 the World Health Organization (WHO) organised a scientific meeting which recommended, ‘Increased concerns regarding risks to public health resulting from the use of antimicrobial growth promoters indicate that it is essential to have a systematic approach towards replacing growth promoting antimicrobials with safer non-antimicrobial alternatives.’ In the newly issued ‘Global Principles for the Containment of Antimicrobial Resistance due to Antimicrobial Use in Animals Intended for Food’, WHO recommends that the use of antimicrobial growth promoters that belong to classes of antimicrobial agents used (or submitted for approval) in humans should be terminated or rapidly phased out in the absence of risk-based evaluations (WHO, 2000).

Regulations

Worldwide, there are great differences in the regulatory control of antimicrobials for therapy, prophylaxis and growth promoting purposes. In US, low doses of Tetracycline and Penicillin are still used as feed additives for prophylaxis and growth promotion without veterinary prescription, while therapeutic antimicrobials are often prescription-only medicines.

Implications to the use of AGP,s

Resistant genes are flowing freely between animal and human bacteria through the food chain which makes the situation more alarming. Of great concern was the possibility that resistance generated on the farm could lead to a loss of effectiveness of key antibiotics in human medicine Although the widespread use of antimicrobials in human medicine undoubtedly is of more importance for the emerging antimicrobial resistance problems in humans, this cannot justify ignorance of potential human health risks related to the use of antimicrobials in food animals. The continuous use of antimicrobials in feed is one of the major sources of over use and mis use of antimicrobials in animal production.

The usage of antimicrobial agents as feed additives is a complex issue with the following implications.

1. Implications for human health
2. Animal health
3. Animal welfare
4. Food safety
5. Environmental aspects
6. Development of production systems
7. Feeding practices
8. Management

Therapeutic use: Antibiotics use is a significant issue in livestock and poultry industry. The animals suffering from infectious diseases are benefited from the use of antibiotics. However, food safety and quality assurance aspects of food products such as injection site lesions, antibiotic residues, and antibiotic resistance of bacteria must be considered. The injection site lesions are the visible tissue damage due to mechanical and chemical irritation caused by the injection as well as inflammatory response to the irritation. Lesions may occur when any drug, vaccine or even saline water is injected into an animal. Injection site lesions increase trim from the carcass and decreased quality of the meat as far as 6 inches from the injection site (Callan, 2003). These lesions may be detected in tissues for years after the injection. Injection site lesions can be minimized but

will never be eliminated. The term 'antibiotic residue' is the small amount of an antibiotic or its breakdown product(s) that remains in or on an agricultural product following treatment with that antibiotic. Every antibiotic approved for food animal use has a maximum residue limit or tolerance level that is established based on consideration of the potential risks to humans exposed to that drug in food. These health risks (Callan, 2003) include allergic reactions (penicillin), bone marrow suppression (chloramphenicol, sulfonamides), nephrotoxicity (gentamycin), carcinogenicity (sulfonamides), or the potential for developing antibiotic resistant bacteria (all antibiotics). Moreover, starter cultures used in cheese and yogurt manufacture can also be affected by antibiotic residues in milk. This may result in considerable economic loss as product batches may be downgraded or discarded (Mitchell, 2005).

Pharmaceuticals in environment / food chain

Antibiotics are used to treat sick animals and to promote growth of healthy ones. These are not fully used by the animals. A major portion i.e.30- 80 percent of antibiotic dose may be excreted as waste because of poor adsorption. Use of animal waste as farmyard manure (FYM) is a common practice in our country. When manure is used to fertilize croplands, antibiotics in the manure may get into the soil and eventually end up in streams, lakes or rivers. Antibiotic residues in the excreta has potential impact on the environment as compared to industrial chemicals, the exposure routes of veterinary medicinal products to the environment are depicted in figure 1.

Veterinary medicines enter the environment by two ways

1. Directly when using the drugs i.e. the unused as waste
2. Subsequent excretion from the animals in urine and faeces.

The dominating pathway of environmental release in the terrestrial compartment is

1. Application of FYM in arable soil.
2. In fish farms, an unknown part of food-pellets coated with or containing the medical compound (most often an antibacterial drug) will not be eaten hence reach the sediment unchanged.
3. Medicines after consumption may be excreted as unchanged compounds or as metabolites and finally reaches the sediment.

Veterinary medicines are widely used for therapeutic treatment of all groups of animals. The group of antibiotics used as non-therapeutic is used as growth promoters especially in pigs, but also in cattle and poultry. Coccidiostat is only used as growth promoters in poultry production. The medicinal mixtures sold as "premixed" drugs are used for therapeutic treatment of pigs, cattle and poultry.

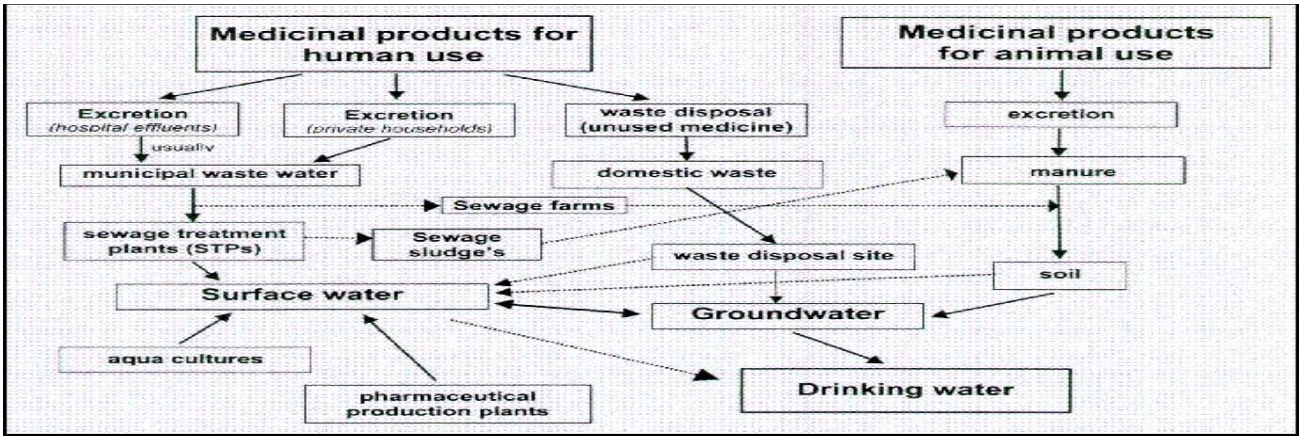

Figure 1: Overview about the pathways by which Pharmaceutical Residues enter the environment (Heberer, T., 2002).

Fate of veterinary medicines during storage

No information is available on the fate of veterinary medicinal products during storage of manure/slurry. There are two types of substances i.e i) hydrophilic and ii) hydrophobic area available in the maure /slurry. Hydrophilic substances dissolve in the aqueous fraction of the manure/slurry while hydrophobic substances adsorb on the particulate matter.

The drug residues present in dung depending on its chemical properties either undergo degradation or leach to the soil in cases of rain. In grazing animals drugs released via the urine immediately reach the soil and if water soluble leach further down to groundwater or adjacent water systems with the soil water. Most drugs excreted by the urine are by nature relatively water soluble, whereas drugs excreted via faeces in general is less soluble.

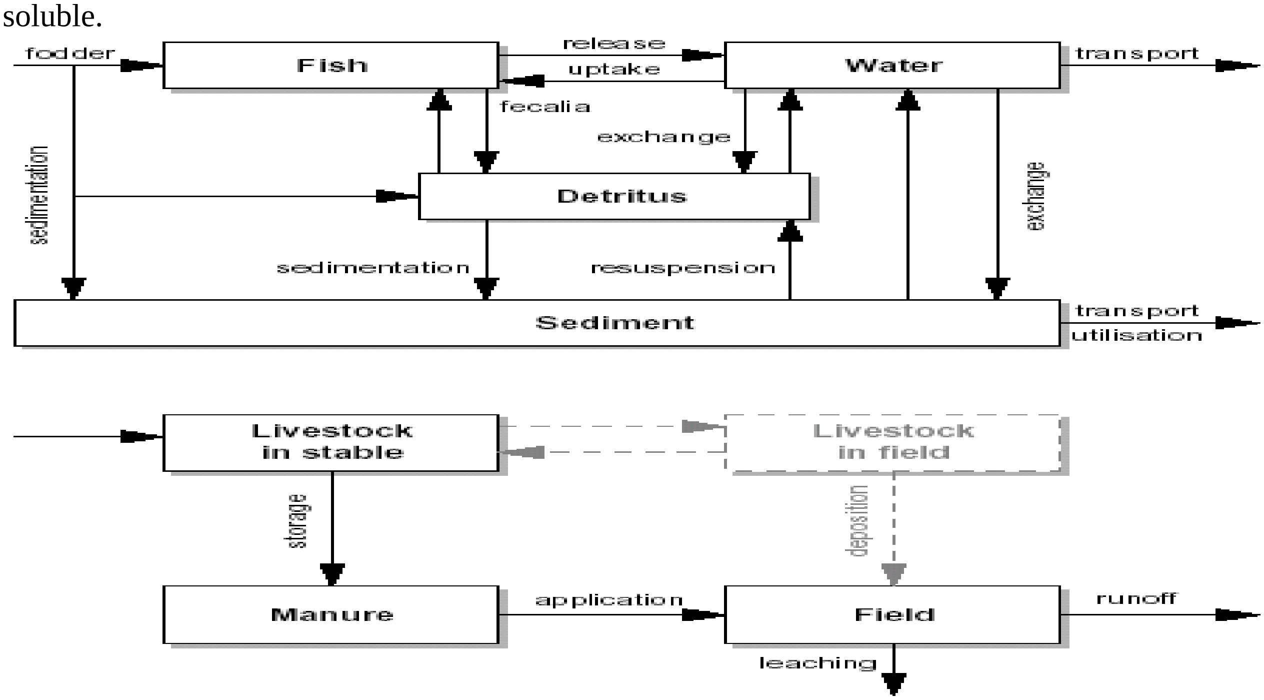

Figure2. Environmental release of veterinary medicinal products used in aquaculture and in livestock production.

ANTIBIOTICS IN SOIL

Soil application of animal and poultry manure is common practice in many parts of the country for its value in supplying nutrients and precious organic matter. Live-stock population in the country is bound to increase in future because of increase in demand of live-stock products including meat in view of increasing purchasing power of the people with increase in income of middle-income group of people in the country. This will result in increase in live-stock population in the country. This suggests that in future still higher amounts of antibiotics will find their way to terrestrial environment through use of manures. There is a tremendous growth in poultry for meeting increased demand for meat and eggs in the country. Continuous use of antibiotic laden animal- and poultry manure can ultimately lead to severe environmental problems in terms of toxicity of these antibiotics to soil micro-flora and –fauna as well as development of resistance in some of deadly bacteria whose control may become impossible with the use of available antibiotics existing to-date.

Land application of manure is a common practice because of its value in supplying nutrients to crops as well as a means of disposing unwanted waste. Spreading of manure/slurry and dropping of excreta by field animals may lead to a run-off of especially hydrophilic substances in cases of subsequent heavy rain.

Microorganisms are essential to life on earth as they are responsible for nutrient cycling, organic matter decomposition and a variety of other functions. Antibiotics, which are designed to kill disease-causing bacteria, could wreak havoc on microbial communities in the soil. Antibiotics might disrupt essential biological activity in the soil. One consequence might be the emergence of antibiotic-resistant bacteria that could infect humans, livestock and wildlife. After the application to animals, antibiotics will eventually enter the environment. In fish farming, antibiotics are given as feed additives, and are directly released into the aquatic environment (Thurman et al., 2002). Approximately 70 to 80 % of drugs administered to fish enters the environment. Antibiotic residues with significant antibacterial activity were found in the sediment of fish hatcheries (Samuelsen et al., 1992).

Of the total drugs administered 40 to 90 % are excreted as parent compounds by animals after medication (Halling-Sorensen et al., 2001; Winckler and Grafe, 2001). The most common antibiotics present in swine, beef, and turkey manures are tetracyclines (oxytetracycline and chlortetracycline), tylosin, sulfamethazine, amprolium, monensin, virginiamycin, penicillin, and nicarbazine (Webb and Fontenot, 1975; De Liguoro et al., 2003; Kumar et al., 2004, 2005). The concentration of these antibiotics varies from traces to as high as 216 mg L^{-1} of manure slurry (Kumar et al., 2005). Concentration of antibiotics in manure excreted from treated animals can be as high as several hundreds of mg kg^{-1} (Migliore et al., 1997; De Liguoro et al., 2003). Soil and water contamination from manure fertilization and at concentrated animal operations has been frequently reported (Rabolle and Spliid, 2000; Hamscher et al., 2002; Boxall et al., 2002). There is concern that residual concentrations of antibiotics in agricultural soil can easily reach levels similar to pesticides if the manure loading for fertilization increases to the kilograms per hectare level (Thiele-Bruhn, 2003).

The antibiotics generally remain stable during manure storage and end up in agricultural fields on manure applications (Boehm, 1996 and Migliore et al.,1995).

Antibiotics And Soil Health

Antibiotics when enter the soil affect its environment by several ways

1. Alter the composition and diversity of indigenous soil microbial communities, which are of fundamental importance for ecosystem.
2. Change function in nutrient cycling,
3. Change decomposition, and
4. Changes in energy flow (McCracken and Foster, 1993; Schmitt et al., 2004).
5. Cause the formation of resistance,(even cross and multiple), in organisms in the soil environment (Al-Ahmad et al., 1999; Sengelov et al., 2003) .

Widespread contamination of veterinary antibiotics exposes humans and animals to a constant low concentration of antibiotics. This threatens the human and animal health by diminishing the success of antibiotic treatment. Evidence has been presented that antibiotic resistant genes from microorganisms in the environment can be transferred directly to humans (Rhodes et al., 2000). Leaching and runoff of antibiotics from manure-fertilized lands is threatening the quality of drinking water (Hirsch et al., 1999; Kolpin et al., 2002).

Though the effects of long-term exposure to low concentrations of antibiotics are not yet clear, the potential danger resulting from veterinary antibiotic contamination to human and animal health cannot be neglected.

Degradation of antibiotics in soil: There are very few studies on reaction of antibiotics in soil. Photolysis, hydrolysis, bio-degradation and binding on to soil particles through adsorption process are some of the reactions of these products that can take place in soil and influence their persistence in soil. They may form complexes with soluble organic materials in the soil and may become more mobile. In this form, they become more mobile and can contaminate even ground water while still in its parent form. Soil pH plays important role in ionization of most of the soil applied antibiotics. Tetracycline group of antibiotics behave as amphoteric compounds in soil. They are stable under low pH conditions but not under basic soil conditions. Sulfonamides, on the other hand, behave like weak acids and can form salts under strong acid and basic conditions. Some of the strongly bound antibiotics in the soil can contaminate the surface waters when these adsorbed antibiotics move with the soil sediments during the run-off losses of soil.

Studies in other countries have clearly indicated that manure from animals fed with antibiotics contains bacterial isolates that are highly resistant to one or more antibiotics as compared to manure from animals which were not fed antibiotics. European Agency for the Evaluation of Medicinal Products has recommended a more intensive environmental safety evaluation of veterinary medicinal medical products if any ingredient or metabolite is present in manure in concentrations > 0.1 mg/kg. WHO of UN in the year 2000, recommended ban on antibiotics as growth promoters in animal feed if these antibiotics are also used in human therapy and in absence of risk-based evaluation. In 2006, EU completely banned the use of antibiotic–growth promoters in animal agriculture. So far, there have been very few studies on the impact of the antibiotics added to the soil through use of manure, sludge and waste waters on the environment and perhaps non in this country. We need to collect precise data on antibiotic use in animal agriculture and the potential reservoir for residual antibiotics in the terrestrial environment in the country. Besides, research work is needed to understand kinetics of biodegradation and potencies of degraded products of various antibiotics in different soils, manures and water wastes. This would help us to better understand the eco-toxicological impacts of various antibiotic residues in the environment. These antibiotics can influence the biological processes in the soil and can affect the organic matter transformation, biological N

fixation, degradation of herbicides/ pesticides in the soil. The purpose of this paper is to initiate discussions to have an overview of the pertinent information about the use of antibiotics in agriculture and their subsequent fate and impact on the terrestrial environment in this country.

Numerous studies have been performed to investigate the degradation of antibiotics in manure (Kuhne et al., 2000; Teeter and Meyerhoff, 2003), soil (Gilbertson et al., 1990; Marengo et al., 1997), and water (Alexy et al., 2004; Boreen et al., 2004). It was recognized that aerobic conditions were always beneficial for the degradation of antibiotics in manure and water (Kuhne et al., 2000; Ingerslev et al., 2001).

Wang et al., (2006) rather suggested that than developing technologies to enhance the degradation of antibiotics in soil and to prevent them from contaminating surface and ground water, a more effective and practical way to reduce contamination from veterinary antibiotics would be eliminating the antibiotics in the manure before its application to agricultural land as fertilizer

There is considerable need for further research into environmental effects such as sorption, mobility, and microbiological changes. This is also the case for other substances which have not undergone valid environmental risk assessment.

The antibiotic dose varies from 3 to 220 g Mg^{-1} of feed that depends on the type and size of the animal and the type of antibiotic (McEwen and Fedorka-Cray, 2002; Kumar et al., 2005). It is claimed that these low quantities of antibiotics encourage the selection of antibiotic resistant bacteria in the environment (Hirsch et al., 1999; Khachatourians, 1998; Boxall et al., 2003).

Persistence in soil

Antibiotics like ciprofloxacin, ofloxacin, and virginiamycin degrade very slowly and may persist in soil in its original form up to 30-80 days while bambermycin, tylosin, and erythromycin completely degrade in a period of one month at temperatures ranging from 20-30^{0}C. Persistence of an antibiotic in the terrestrial environment is the key factor determining its environmental impact. Most of the antibiotic residues in manure generally remain stable during manure storage until its application to agriculture fields. On repeated application of antibiotic-laden manure to the soil result in significant concentration in the soil making them potential source to contaminate the terrestrial environment. Persistence of antibiotics in soil is determined by a number soil and environmental factors. Properties of antibiotics also influence their persistence in soil. Properties that matter are, their photo-stability, binding with soil particles, adsorption on soil colloids through surface reactions, water solubility, and their biodegradation. They may form complexes with soil organic matter and may become more mobile. In this form, they can move faster in the soil and can contaminate even the ground waters while still retaining its original parent structure.

Effect of Soil pH

Antibiotics vary in their molecular structure, molar mass and their physico-chemical properties. These properties will determine as to how they will ionize in soil as a result of variation in soil pH. Soil pH plays important role in ionization of most of the soil applied antibiotics and their dissociation constant values, pKa determine the ease of their ionization in soil. The anti-microbial properties of the antibiotics in soil are determined by their active functional groups.

Most of the antibiotics belonging to tetracycline group are amphoteric in nature, which are stable under acid conditions when the soil pH values are below 7.0. These compounds can form chelate-complexes with divalent metal ions like Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, and $Cu2^{+}$ etc. Sulfonamides, on the other hand, have two pKa values, they behave as weak acids and form salts both under acid and basic conditions. Aminoglycosides are polar compounds, they are highly water soluble. They can easily move with the percolating water and can contaminate the ground water resources, but they are photo-degradable, therefore, easily decompose when subject to sun-light. Penicillin belongs to β-lactan class of antibiotics and its antibiotic effect is due to its β-lactan ring. It is stable under a wide range of pH values from strong acid to strong alkali conditions. This speaks of its high stability in soil in original parent molecule and is a potential health hazard. Fluoroquinolones, on the other hand resist break down through hydrolysis and therefore are highly stable in soil solutions.

Adsorption reactions

Antibiotics in soil can be retained on the mineral and organic colloids through the process called adsorption. In this form, they are less liable to degradation forces and also less potent towards its targets in soil. Soil colloids carry permanent negative electrical charges which attracts the oppositely charged ions. There could be positive charge which is not permanent but pH dependant. Antibiotics that can ionize in soil to furnish charged ions can be retained on soil colloidal surface through the adsorption process. How strongly the antibiotics will bind in the soil is determined by the value of slope of the adsorption isotherm obtained from the relationship between the adsorbed concentration vs. solution concentration of an antibiotic in soil at equilibrium. Antibiotic compounds with higher values of slope, which is also called distribution coefficient, and referred to as *Kd,* are strongly bound with the soil and they are less mobile, while compounds with less *Kd* value are less strongly bound and more mobile in the soil. The later group of antibiotics can be easily transported to contaminate the ground water or surface waters. Strongly bound antibiotics, can, however, be transported mainly to surface waters with the sediments during runoff losses of soil. Some of the antibiotic compounds can form complexes with soluble organic matter in the soil, which increase their mobility, in this form they easily find their way to contaminate ground waters even though under ordinary conditions, they are strongly bound to soil solids and thus highly immobile. In highly developed dairy farming countries like Germany, sulfamethoxazole concentration as high as 40ng L^{-1} have been reported in almost 10% of the ground waters tested. Sulfonamides have little adsorption tendency and also do not form immobile complexes in soil, therefore, the antibiotics are very mobile under similar conditions, while tetracycline is likely to contaminate surface waters, sulfonamides are strong contaminators of ground waters as well as surface waters.

The tenacity with which the antibiotics are held on the soil solid surfaces will also be determined by such properties as pH, soil clay content, and soil organic matter content. In acidic soils with pH below 7.0, basic antibiotics acquire a proton and become cation ($-NH_3^+$), while acidic antibiotics remain unionized. In alkaline and alkali soils, basic antibiotics remain unionized ($-NH_2$), while acidic antibiotics get ionized ($-OOC^-$). Amphoteric antibiotics like tetracycline which is most widely used in animal feed, may exist as anions and as cations depending on pH of the medium. Cationic antibiotics bind to the soil particles through ionic interaction, while acidic and amphoteric antibiotics may bind the soil through non-ionic interaction.

Effect of type of clay mineral

Depending on the reaction of antibiotics with the clay minerals, antibiotics can be divided into 3 groups:

I. Strongly basic - Streptomycin; dihydrostreptomycin; neomycin and kanamycin.
II. Amphoteric - Bacitracin; aureomycin; and tetramycin
III. Acid - Penicillin
IV. Neutral - Chloromycetin and cyclohexamide

In soils dominated by montmorillonite or illite or kaolinite, clay mineral reacts with the first two groups (strongly basic and amphoteric) of antibiotics to form complexes. But acidic and neutral antibiotics are adsorbed only in soil which dominantly contains montmorillonite type of clay mineral; still the tenacity of adsorption is relatively weak. On an average, the amount of antibiotics adsorbed by clays varies from 9 mg g^{-1} for kaolinite clays for strongly basic antibiotics to more than 300 mg g^{-1} in case of montmorillonite clays for amphoteric antibiotics. Strongly basic antibiotics are so strongly held on the clay surface of montmorillonite, vermiculite or illite minerals, that they are virtually un-releasable as assessed fro bio-assay studies. In kaolinite, however, where it is not held that strongly, there is some release in case of streptomycin and dihydrostreptomycin. But in case of amphoteric antibiotics, there is easy release from all kinds of clay minerals. Streptomycin which is commonly used as growth promoter in swine is adsorbed strongly on the soil particles, highest in clay and lowest in sand fractions. Wide variation in tenacity of their adsorption on soil exchange sites is apparent from a vide range of observed sorption distribution coefficient *Kd* from 0.2 to as high as 6000 L kg^{-1}. Clay adsorption is the main reason, role of organic colloids in this respect is not significant, weakly adsorbed antibiotic compounds such as metronidazole and olaquindox are more mobile and can leach with percolating water. However, strongly adsorbed oxytetracycline and tylosin percolate the least in the leachate.

We can see that overall, the affinity of many of the commonly used antibiotics as growth promoters is quite high to soil particles which is indicative of the fact that most of the mobility of these antibiotics in terrestrial environment is probably due to run-off losses of antibiotic-laden sediments to surface waters from fields where antibiotic laden manures are applied.

Effect of type of Soil

Soil type, whether loam, silt loam or sandy loam matters in the persistence of antibiotics in soil, for example, it has been found that ciprofloxacin was mineralized to CO_2 less than 1% in all the three soils in 80 days of the incubation studies. Strong binding of this antibiotic was sited as the reason for its slow degradation. Half life of ceftiofur was more than 49 days in sand and only 22 days in clay loam. This suggested that soil type or soil texture plays prominent role in the persistence of the antibiotics in soil. Half-life of oxytetracycline in marine sediments at a depth of 5 to 7 cm was more than 300 days as compared to 87 to 173 days for virginiamycin in sandy soil. This shows that antibiotic persistence in soil is determined not only the soil type but also soil depth. Antibiotics can persist for longer periods if they are lodged in sub-surface soil layers and deep in waters away from sunlight.

Effect of soil Temperature

Most of the degradation process of antibiotics in soil is mediated by soil micro-organisms, therefore persistence of these compounds in the soil are affected by all those factors that affect the activity of microbes in the soil. Soil temperature is an important

factor in this respect. As the temperature decreases from the normal range of 25-30^{0}C, persistence of antibiotics increases. At 30^{0}C, 44% of chloretetracycline and 23% of bacitracin remained in the soil after 30days of their application. However, when temperature decreased to 20^{0}C, 88% of chloretetracycline, 33% of bacitracin, 25% of erythrocin remained in soil. At 4^{0}C, almost all chloretetracycline, erythrocin and bembermycin persisted in soil. It is very likely that under north-western Indian conditions, antibiotics finding their way to the fields with manures during *Kharif* season, a rapid decomposition may eliminate it from the soil due prevailing moderate to high temperature, but during *Rabi* eason, when atmospheric temperature is low, antibiotics applied through manures may remain intact in their original parent form over the winter season.

Eco-toxicological Impacts

Antibiotic laden poultry and animal dung manures are mainly applied to the soil as source of nutrients for crop plants, how it affects soil fauna and flora, enzymatic activity and nutrient-cycling needs greater emphasis for investigation. These impacts could be direct such as antibiotic toxicity to soil microbes and indirect effect such as nutrient availability due to changed microbiological activity in soil. Decomposition of organic matter in the form of manures and crop residue depends on various microbial processes, which, in turn depend on type and population of micro-organisms in soil. Repeated application of antibiotics in soil through manures may adversely affect decomposition process by curtailing the microbial population itself and also by allowing only the antibiotic-resistant microbes to flourish which may affect decomposition differently. Oxytetracycline or chlortetracycline-fed animals have been found to result in the manure/feces which when applied to the soil result in more evolution of CO_2 than the feces of the animals fed with rations without antibiotics. It has also been reported that feces or dung from the animals fed with antibiotics contained higher proportion of easily decomposable/degradable C compounds. Ionophore antibiotics such as monensin favours growth of Gram-negative bacteria in animal gut, which, in turn changes the fermentation dynamics, improves dietary protein efficiency, and results in less methane production, as much as 25%. The effect of monensin in decreasing feed intake by animals and reduced excretion of nitrogenous compounds such as ammonia and also methane are environmentally beneficial. Similarly tylosin addition into the pig feed can result in reduced N excretion by as much as 10%. This means if we ban the use of antibiotics in the animal husbandry, there could be 7-10% increased loading of environmental N.

Impact on non-target micro-organisms

Antibiotics like streptomycin-laden manure when applied to the soil has been found to decrease bacterial count in soil up to 50-75% over several months. The effect will depend upon the nature of bacterial population, a more resilient population shows effect only for a limited period as compared to non-resilient population. Streptomycin has been specifically found to adversely affect the nitrifying bacteria as NO_3-N concentration in the soil decreased significantly with the application of streptomycin-laden manures. Gram-negative bacteria like *nitrosomonas spp* are responsible for nitrification in soil. Therefore, broad spectrum antibiotics like tetracyclines, aminoglycocides, and sulfonamides are expected to inhibit the nitrification process in soil, however, narrow spectrum antibiotics such as sefdiazine, oxolinic acid, and tylosin simulated the nitrification process. Veterinary antibiotics may also inhibit SO_4 reduction as well as OM decomposition. Build up of tylosin in soil through repeated application of tylosin-loaded manure in soil can cause selective pressure on soil bacterial population, shifting bacterial

communities from Gram-positive to a Gram-negative; the consequences of such a selective change in population are well known.

Emergence of anti-microbial resistance

Widespread use of and their subsequent release into the environment may lead to the selection of antibiotic-resistant bacteria in the environment. There has been a frequent observation on shortening of the time between introduction of a new antibiotic and development of resistance of the targeted microbial species. If spontaneous mutations were the only cause of antibiotic resistance, it would have been restricted to only a few bacterial species amongst the hundreds of billion in one antibiotic-treated host, and would not be the epidemic problem as it is to-day. A higher degree of vancomycin resistance (60%) in various enterococci isolates from the broiler fecal samples has been reported. Similarly, a high level of resistance among Gram-positive and Gram-negative isolates from various meat products have been observed for such antibiotics as penicillin, erythromycin, sulfamethoxine, tetracycline, ceftibiotics, and gentamycin. Therefore, animal manure containing such antibiotics can cause elevation of resistance in soil bacteria after manure application year after year. The studies have shown that development of resistance is quite rapid, it has been found that within 3 weeks of antibiotic feeding; more than 70% of fecal bacteria were resistant to penicillin and tetracycline. All these studies show that antibiotic feeding of animals provides an environment that selects resistant strains and perhaps also encourages the transfer of genetic information from even unrelated bacterial species.

Every time some drug becomes ineffective against resistant bacteria, it adds to the cost of treatment. Discovery of new drugs is not only expensive but buys us only a short time. It is thus important we should look for safer growth promoters and use antibiotics to a bare minimum especially those antibiotics that are used by both animals and humans.

Resistance in food-borne pathogens

If there is development of resistance in this way into the food-borne pathogens, then it really becomes a problem, because these infections can become difficult to treat with traditional antibiotics, thus threatening human and animal life. The reports of several incidences of infection by multidrug-resistant *Salmonella typhimurium DT 104* in the past decade is the result of such a possibility. Out of 120 isolates of *E.coli* from animal food showed that around 20% were resistant to multi-antibiotic drugs. Similarly, a wide range of tetracycline-resistant genes of *E.coli* have been observed as isolated from human and animal sources. A recent study showed that not only Gram-negative enterobacteria but also Gram-positive bacteria are the major source of antibiotic-resistant integrons in animal litters. In U.S.A., it has been found that more than 40% of bacteria collected from surface waters were resistant to one or more antibiotics. Similarly, 54% of the coliform isolates obtained from Korean river were found resistant to at least one antibiotic. Similarly in Greece, 20% of the *Salmonella* samples isolated from surface waters were resistant to antibiotics. Tetracycline is commonly used antibiotic in animal agriculture. Selection of tetracycline-resistance occurs in the animal gut and these resistant bacteria are released into the environment when the wastes from these animals are land-applied.

Thus, the use of antibiotics any where in the world can increase antibiotic resistance somewhere else, following the simple ecological principle that “every thing is connected to everything else”. The transfer of resistant bacteria is not restricted to a particular country or a continent, because animal food products are traded world-wide. This

suggests that prevention of further spread of resistance from the bacterial communities via animal products required global regulations.

Antibiotics and Plant Uptake

Until recently, research on antibiotic use has been mainly directed toward their beneficial and adverse effects on the end user, humans and animals. However, there have been relatively few studies on the effect of these antibiotics on their uptake by plants from manure-amended soils. Consumers may unknowingly be ingesting some of these antibiotics when they eat vegetables grown on manure-applied lands.

The adverse impacts of ingesting antibiotics present in plants by humans are not known at this stage. A few adverse impacts of consuming antibiotics in fresh vegetables (sweet corn) and fruits are speculated in the following discussion:

- Allergic or toxic reactions: Some of these antibiotics when ingested by humans, especially children, may cause serious allergies or may be toxic (Patterson et al., 1995). There may be some interaction effects from simultaneous ingestion of two different antibiotics. It has been shown that some of the macrolide antibiotics present in animal feed have interacted with other antibiotics like monensin and have resulted in toxicity of monensin and the death of affected cattle (Basaraba et al., 1999).
- Antibiotic resistance: Antibiotics present in plant materials ingested by humans may provide resistance to human pathogens thus resulting in illnesses that may be difficult to cure with presently available antibiotics. This can be a serious threat.it has been shown that resistance of gut bacteria to antibiotics increased in cases when fed increasing concentrations of penicillin in contaminated waste milk as compared to dairy calves fed with noncontaminated milk (Selim and Cullor, 1997; Langford et al., 2003). Small amounts of tetracycline can act as a catalyst in triggering the horizontal gene transfer between different bacteria (Shoemaker et al., 2001).

This increasing resistance may be of concern both for human and animal health if antibiotics are present in food crops. Our knowledge regarding the implications of manure antibiotics on the terrestrial environment and impacts on human health is limited. There is an urgent need to study (i) the fate of different antibiotics present in manure, (ii) which antibiotics and their degradation products may be taken up by plants grown on manure amended soils, (iii) whether or not antibiotics present in food degrade when cooked, and (iv) whether or not antibiotics or their degradation products are still bioactive to impart antibiotic resistance to gut bacteria or cause adverse immunological reactions in humans.

ALTERNATIVES TO AGP's

Ban on use of AGP's has created the need to explore the alternatives that can improve the general health status and enhance the immunity to fight against disease (Bosi & Trevisi, 2006). This increased the interest of nutritionists toward natural substances like botanicals, herbs, nutraceuticals, enzymes etc. Some of the potential alternatives tried in the past with their relative effectiveness are mentioned in table 7. During the recent past research activities were focused on the area of use of phytogenic feed additives and botanicals / herbs but still the mode of action of these additives is not very clear. This area needs much more studies and research

Table7. Potential antibiotic alternatives

Sr. no	Compound	Species.	Relative effectiveness	Comments
1	AGP		+++++	Standard for Comparison
2	Zinc oxide	Pigs	++++	Dose of 2000-3000 ppm 1-2 weeks post weaning. Decrease scours and improve performance
3	Copper sulphate	Pigs	+++	Dose 200-250 ppm improves performance. Maximum effect in nursery
4	Plasma protiens	Pigs	+++	Increased feed intake and improved performance. More effective in unhygienic conditions
5	Egg yolk antibodies		++	Limited data.
6	Organic acids	Pigs	+++	Most effective in newly weaned pigs. Formic acid is more effective
7	DFM's	Pigs & poultry	++	Promote beneficial bacteria in the gut
8	Prebiotics	Pigs & poultry	++	Promote beneficial bacteria in the gut
9	Enzymes	Pigs & poultry	++	Improve digestibility and gut health
10	Bioactive peptides		++	Some peptides have antibiotic activity and can have potential benefits
11	Botanicals (Herbs/Spices)		+	Many potential compounds May be beneficial. More research needed
12	Essential oils		+	More research needed
13	Fermented liquids feeds		+	Organic acids are produced which maintain gut pH

.

Several foods besides containing nutrients also contain certain compounds that enhance the production by providing either the nutritional balance, improving the metabolism or preventing the disease. Moreover at the same time there is increased interest over the food safety, environmental contamination and the general health risks which have made *natural* the norm, promoting the trend towards alternative strategies to manage and feed the animals and birds without reliance on antibiotics. Such types of foods are labelled as adaptogens, dietetics, nutracines, nutraceuticals or multifunctional additives.

ACID BARRIER – CENTRAL AND HIND GUT

Prevention of harmful bacteria from entering the intestines by the oral route is the first line of defence. Acidic conditions in the stomach due to the secretion of hydrochloric acid acts as a powerful antimicrobial barrier. This mechanism is inadequately developed in the newly weaned piglets. Lactic acid originating from the fermentation of lactose by lactic acid bacteria (naturally occurring and probiotic additives) is helpful but limited by the relatively small amount of bacterial activity in the stomach and proximal small intestine. Anything that increases acid production post weaning (Prebiotic SCFA, Probiotic Lactic Acid) can enhance antimicrobial competence and improve the barrier to orally acquired pathogens.

The gastrointestinal (GI) tract of vertebrate animals contains a species-diverse group of microflora. Bacteria, particularly gram-positive, predominate (Savage, 1977; Mackie et al., 1999). As many as 500 bacterial species exist in the GI microflora, with numbers up to 1010 to 1012 bacterial cells/g of colonic content or feces (Moore and Holdeman, 1974; Savage, 1977; Lee, 1984; Jensen, 2001). These numbers are consistent with the estimation that bacterial cells out number host cells by 10:1 (Gaskins, 2001). The bacterial population influences a variety of immunological, physiological, nutritional, and protective processes of the GI tract and exerts profound effects on the overall health, development, and performance of monogastrics. Indeed, experiments comparing conventionally reared versus sterile (germ-free) animals have demonstrated that commensal bacteria play important role in organ, tissue, and immune system development, as well as providing a variety of nutritional compounds (Gaskins, 2001; Snel et al.,2002). The benefits imparted by normal microflora come at a great cost to the animal, even under ideal conditions. The commensal bacteria compete with the host for nutrients, secrete toxic compounds, and induce an ongoing immune/ inflammatory response in the GI tract. All of these costs negatively impact animal health and performance. Two important areas for future research are 1) to determine the optimal microflora for animal health and performance under commercial growth conditions in other words, to discover the microflora that maximize the benefits while minimizing the costs and 2) to develop dietary and other interventions to foster development of this microflora.

BALANCE OF GUT MICROFLORA

A delicate balance between the beneficial bacteria (Lactobacilli, Bifidobacteria and Eubacteria) and the potential pathogenic bacteria (E Coli, Salmonella, Staphylococci, Listeria, Shigella, Veillonella, Brachyspiro (Serpulina), Clostridia and Coliforms) exists in the gut. A ratio of 9:1 between beneficial and pathogenic bacteria is considered to be ideal. But the factors like drug administration, stress, environmental and manage mental changes, spoiled feed or change in gastric pH can alter this ratio. The pig monitors what bacteria are within its gut and reacts to what is there. Pigs grow faster or slower according to what it 'sees' in its gut!

The digestion efficiency in poultry and pigs depend upon the micro organisms which live naturally in its digestive tract. The microbial population present in the intestine of chicken comprises more than 90% of all the living cells in the bird. At least five hundred bacterial species colonise the pigs intestine (10^{11} cfu /g intestinal contents). This is ten times more cells than the number of cells in the pig body. The intestinal microflora have important and differing effects, including regulation of epithelial cell turnover, competition for ingested nutrients, modification of digestion, competitive exclusion of pathogens, metabolism of mucus secretions and modulation of mucosal immunity (Hooper et al, 2002). To make the environment conducive for the beneficial bacteria pre and probiotics are added in the feed. These are beneficial nutritional modifiers for monogastrics.

BACTERIAL METABOLISM

The main end products of bacterial carbohydrate metabolism are acids, short chain fatty acids (SCFA) mainly acetic, propionic and butyric acids. SCFA are weak organic acids with bacteriostatic properties in common with the organic acids used as preservatives. SCFA play an important role in the prevention of potentially harmful bacteria escaping the stomach and migrating forward through the small intestine, but more important is the reverse flux of harmful bacteria from hind gut to small intestine. The presence of fermentable carbohydrates in the pigs diet reduce protein fermentation, reducing toxic

substances such as ammonia, amines, skatol and indole. Higher butyrate concentrations contribute to a healthier intestine because butyric acid is a strong stimulator of the gastrointestinal cell growth, not only for the colonocytes, but also for the enterocytes of the small intestine, (Pouillard P, 2003). Immune cells form part of the intestinal epithelial lining who's function is to monitor, react and coordinate a response to the components of the intestinal microflora. Prebiotics and probiotics increase the chances of a favourable response to the monitoring process, minimising immune activation with its highly beneficial impact on appetite and nutrient partitioning to growth. Growth responses to Pre and Probiotics achieve statistical significance during the first 14 days after weaning of piglets which confirms they can be fast acting in their influences. (Corrent, 2002).

NUTRACEUTICALS: The term nutraceuticals is a combination of nutrients and pharmaceutical. Their use is not a newer concept, but it is an example of history which is repeating itself (Dzanis 1999).

Table 8. Difference in nutraceuticals etc

Term	Definition	Source
Veterinary Nutraceuticals	A non drug substance that is produced in purified or extracted form and administered orally to provide agents required for normal body structure and function with the intent of improving health and well being of animals.	Booth (1997)
Dietary Supplement	Any substance …that contain a vitamin, a mineral, an herb or other botanicals, an amino acid, a dietary substance for use to supplement the diet by increasing the total dietary intake, or a concentrate metabolite,constituent extract or combination of any of the previously mentioned ingredients	FFDC Act (1998)
Drug	Article intended for use in the diagnosis cure mitigation treatment or prevention of disease in man or other animals and articles (other than food) Intended to affect the structure or function of the body in man or other animals.	FFDC Act (1998)
Botanicals	Drug made from part of plant, i.e. root, leaves, bark etc, essential oils or any of a class of volatile oils obtained from plants, possessing the odour and other characteristics properties of the plant, used chiefly in manufacture of perfumes, flavours and pharmaceutical extract after hydro distillation	Webster Dictionary of English (1989)

Word 'Nutraceutical' was first coined by Stephen Defelice, the founder Chairman of "Foundation for Innovation in Medicine (FIM)". Booth (1997) defined veterinary nutraceuticals as a non drug substance that is produced in purified or extracted form and administered orally to provide agents required for normal body structure and function with the intent of improving health and well being of animals.

Recently, Sarah (2003) reported that nutraceuticals must improve the performance effectively & economically, with little therapeutic use, without causing cross resistance to other antibiotic at actual use level, without involving with transferable drug resistance, without causing any deleterious disturbance to the normal gut flora and should not create

environmental pollution. Moreover these must be non toxic to the animals and its handlers.

Nutrition based health (NbH):- A new concept, according to this concept feed and feeding programmes must be designed to reduce stress and to assist the animals in resisting disease challenges (Adams, 2005). Judicious use of various nutrients and bioactive feed components like acidifiers, antioxidants, bacterial inhibitors, enzymes, flavours etc. to support animal health is the right approach of Nbh.

A term **'pronutrient'** i.e. a micro ingredient included in the formulation of animal feed in relatively small amounts with specific physiological and microbiological functions different from any other nutrient is included in the feed additive list. Many active ingredients from plants must be considered pronutrients due to their effects against the colonization of different pathogenic organism and stimulation of beneficial bacteria eg zinger for the treatment of dysentery.

Broadly nutraceuticals / natural therapy is classified as Herbs & Botanicals, Antioxidants (Vitamins C, A, beta carotene), Enzymes and Prebiotics and Probiotics or Direct Fed Mcrobials (DFM).

HERBS/BOTANICALS: Vegetative parts of the plants (leaves, bark, fruit, roots, seed and their extract) containing a variety of chemical compounds that are used as body restoratives are called herbs. While drugs are made from any part of plant (root, leaves, bark etc), essential oils or any of a class of volatile oils obtained from plants, possessing the odour and other characteristic properties of the plant, used chiefly in manufacture of perfumes, flavours and pharmaceutical extract after hydro distillation (Webster Dictionary of English, 1989)

These chemical compounds are active in altering the physiological and biochemical processes in the body. Herbs and spices have compounds with antibacterial effects for example garlic contain allicin and ajoene which exhibits broad spectrum anti microbial properties (Naganawa et al, 1996) and is effective in reducing cholesterol of liver, breast and thigh muscle (Kopnjufca et al, 1997). Another example is of Yucca Schidiger which improve growth & FCR (Headon et al 1991).

Botanicals / herbs help in improving the performance by several ways.

- Reducing the stress associated with handling, transport and poor health by providing nutrients and or active principles which act as anti stress agents.
- Being adaptogenic manage stress and improve egg production in birds.
- Increase the feed consumption due to the flavours present
- Ensure the normal gut functioning.
- Improve the digestion by activating digestive secretions.
- Improve the feed conversion efficiency there by growth and production.
- Improve the liver functioning.
- Act as toxin binder and reduce the risk of mycotoxicosis.
- Normalize the kidney functioning.
- Improve the immunity as an immune modulater.
- Antioxidant
- Act as coccidiostat and anti helminthic
- Stimulate endocrine system
- Stimulate intermediate nutrient metabolism
- Stabilize gut environment.
- Ameliorate the effect of ANF's present in the feed.
- Used for the treatment of bacterial (Yuan et al, 1993), viral (Yu & Zhu, 2000) and parasitic diseases (Pang et al 2000).

- Reducing ammonia and other noxious gases in the GI tract through their binding to the saponins and excreted in the excreta and
- Reducing the ascitic mortality in broilers (Menocal, 1995).

These properties of various herbs are due to the active secondary metabolites which belong to class of isoprene derivatives, flavonoides and glucosinolates. Intercation between different active components within and between extract may have either cummulative or antagonistic effect. Use of herbs in poultry and pig feeds are now gaining momentum as it claim to have no side effect, safe and eco friendly. A term botanical / natural broiler/pig can be used when only botanical / natural materials are used for enhancing performance and prevention of disease. Sikka and Singh, (2007) recently reviewed use of some herbs in poultry feeds.

The herbs / botanicals through their flavour affect the feed intake and secretion of digestive fluid and stimulate endocrine system and intermediate nutrient metabolism by their active principles. Botanicals in some research are proved equally beneficial as antibiotic growth permotors. Some botanical extracts have both positive and negative effect on the gut micro flora. This nature of botanicals can be useful in the stabilization of gut environment.

Intercation between different active components within and between extract may have cummulative or antagonistic effect. Use of herbs in poultry feeds is now gaining momentum as it claim to have no side effect, safe and eco friendly. Herbs can also be used to ameliorate the effect of ANF's present in the feed. Besides herbs can be used in the treatment of bacterial (Yuan et al, 1993), viral (Yu & Zhu, 2000) and parasitic diseases (Pang et al 2000).Many herbal tonics like Liv 52 (Joshi & Kumar, 1987), Livol (Pradhan, et al. 1987), Ayucal (Jadhav et al, 1999), Livfitvet (Gopinath et al, 2001), Stressroak (Pradhan 1995), livfit (Devgowda & Aravind, 1996), Zeetress (Roy et al, 1996) are well tried in poultry for various beneficial effects. Birren kott et al (2001) reported the reduced northern fowl mite infestation with the topical application of garlic in laying hens.

Activity of herbs: Do the herbs have always the same activity? No, the desired activity of herbs is not always same due to variability of the composition of plant secondary metabolites, environmental conditions, different harvesting time, stage of maturity, method of extraction and conservation, anti nutritional factor and nature of diet in which it is supplemented because it have to compete with nutrients present in the feed.

PREBIOTICS: Prebiotics are short chain non-digestible oligosaccharides with 2-10 units of monosaccharide present in feed ingredients. These are commonly found in soybean and rapeseed meal. Legumes, cereals and yeast cell walls contain respectively α-galactooligosaccharides (GOS), fructooligosaccharides (FOS) and mannanoligosaccharides (MOS). Lactobacilli, Bifidobacteria and Eubacteria selectively ferment some prebiotics. Whilst being poorly utilised by the potentially harmful bacteria listed above. Prebiotics modify the gut microbial population balance by promoting the growth of beneficial flora in the intestines (Gibson et al, 1996,2004, Flickinger & Fahey 2002) thereby providing a healthier intestinal environment. These are not easily digestible and provide competitive advantage to favourable bacteria, inhibiting the colonization of harmful microbes and promoting the beneficial ones.

It is generally accepted that high villi : crypt depth ratios are indicators of a healthier and more efficient intestinal mucosa. Prebiotics have a beneficial effect on the gut integrity especially in the distal end of small intestine, the area with the greatest levels of

fermentation. In a recent experiment, it was observed that ratios were enhanced in distal area, with enhanced fermentation along the entire small intestine (Decuypere, J, 2003).
Through a variety of mechanisms prebiotics are thought to increase resistance to infection. Various proposed modes of action are summarized below

- Enhancement of the physical barrier (modulation of paracellular permeability, mucosal trophic action).
- Improved functional barrier (mucosal immunity).
- Competitive adhesion to epithelial receptors.
- Increased SCFA production along the gastro-intestinal tract
- Inducing a shift to a more saccharolytic (carbohydrate fermenting) flora.
- Reduction of intestinal pH .
- Reducing the colonization of harmful bacteria.
- Excreting harmful bacteria
- Competitive exclusion (colonisation resistance).

Galacto-oligosaccherides (GOS), Mannanoligosaccharides (MOS), Fructo-oligosaccharides (FOS) are frequently used in poultry (Ishihara et al 2000, Zhang et al, 2003) diets. Harmful bacteria attach themselves to both the FOS and MOS and are excreted. FOS, derivative of inulin, stimulate the growth of Bifido bacteria, improve the mucosal morphology of the colon (Howard et al 1995) and inhibit the growth of pathogenic microorganism such as clostridia and salmonella (Wang & Gibson 1993). Chen et al (2005) revealed the increase in egg production and feed efficiency of layer with the use of dietary oligofructose and inulin. The inulin is required for the growth of Lactobacilli (Gibson, 1999). FOS has been reported to improve growth in the weaned pigs by 5.1 % and feed efficiency by 2.0% (Mul & Perry, 1994) On the other hand MOS found to improve daily weight gain by 7.4% and feed utilization by 5.2 % in nursery pigs (Vender beke, 1997) by improving the structural integrity of G-I-tract. Spring and Privulescu (1998) revealed that oligosaccharides stimulate the secretion of cytokine and there by enhance the immune system of the pig to resist pathogenic bacterial challenge.

PROBIOTICS: The live microbial food supplement which when fed improve the intestinal microbial balance of the host are called probiotics or Direct Fed Microbials (DFM's). Probiotics improve the survival with better growth, better-feed conversion and inhibition of diarrhea in piglets (Jin et al 1996). Lactobacilli, Streptococci, Bi-fido bacteria, *Bacillus, Bacteriods, Pediococcus, leuconostoc, Propionibacterium*, and some yeast (*Saccharomyces cerevesiae*) and fungi (*Asperzillus oryzae*) are commonly used DFM's. B *Subtilis* and B *licheniformis* are commonly used in nursery pig rations as they are spore forming and are able to resist the environmental conditions of high temperature and moisture occurring during the pelleting process.

Supplementing probiotics

Probiotics can be administered by two routes viz. through drinking water and by mixing in the feed. Probiotics should be given once or twice daily, after which the bacteria should establish itself in the alimentary canal and replace disease-promoting micro-organisms but results are not convincing. Furthermore, it is practically impossible that probiotic bacteria could establish themselves in a stable alimentary canal system. Therefore these must be added to the feed on a daily basis.
Use of probiotic bacterial cultures have greater effect during the early stages of growth, when, the gut is sterile and when the alimentary flora of pigs are unstable, viz after weaning and subsequent to an extended period of treatment with antibiotics. Probiotics,

improve health and growth by modifying intestinal microbial balance by several ways given below.

- Competitive exclusion,
- Adhering to intestinal mucosa (Jonsson and Conway, 1992)
- Preventing attachment of pathogens, (Green & Sainbury, 2001)
- Production of antimicrobial compunds (Hentges, 1992) such as bacteriocins and organic acids,
- Competition with pathogens for nutrients (Freter,1992),
- Stimulation of intestinal immune responses (Perdigon and Alvarez,1992),
- Affect the gut permeability
- Increase uptake of nutrients (Stavric et al, 1995; Mulder et al, 1997; Lee et al, 1999).

Some bacterial cultures when fed in single or multiple (few doses) to newly hatched birds establish an intestinal flora quickly and it prevents colonization by pathogenic bacteria. For example lactobacilli acidophilus produces lactocidin which has antibacterial effects on E coli. Lactobacilli modify gut pH, competition for nutrients and absorption sites, boost cell immune response, inhibition of bacterial growth by hydrogen peroxide production and cell signaling to turn off pathogenic function (Fuller,1999). Competitive Exclusion(CE) preparations are not always pure cultures of bacteria and their microbial composition may not be completely known. Few CE cultures have proven effective in protecting chicks from *Salmonella* infections.

Interest in the use of probiotics in poultry and pig diets is to curtail sub-therapeutic doses of antibiotics in feed. Like antibiotics, probiotics appear to have a more pronounced effect on farms where housing and hygiene are not optimal (Mulder et al, 1997; Thomke and Elwinger, 1998). Supplementation of probiotics containing *Lactobaccilus acidophilus*, *Streptococcus faecium* and yeasac @ 0.025% in the diets of broilers were found to be beneficial in early stage of growth. In broilers supplementation of yeast culture at 0.1 % level increased the body weight and performance (Tollaba et al 2004) due to quantitative and qualitative alteration in the digestive tract flora with better nutrient utilization (Sara et al 2003).Use of combination of several strains at a time improved the weight gain and feed efficiency in broilers (Mazurkiewicz et al 1992) and in chicks (Nguyen et al,1998).. Feeding of mixture of S. *cerevisae*, L.*bulgaricus* and S. *thermophilus* did not show any effect on the production of layers (Svetic et al 1996).

Dietary supplementation of probiotics results in no effects on growth (Bold et al, 1993; Yadav et al, 1994) or have better growth performance (Couch, 1978, Arendz 1981), with improvement in feed efficiency and low mortality during finishing (Bhatt et al, 1993), increased immunity and carcass characteristics (Panda et al, 2005) and can also replace the anti microbial compounds in the broiler chicks (Ramarao et al, 2004). In layers improvemnet in egg production and feed efficiency have been reported by Nahashon et al (1993), Mohan et al (1996).

In pigs the intestinal microflora is capable of resisting the establishment of certain intestinal pathogens (Lopez & Marquez 1994). Bera & Samanta (2005) fed probiotics (alone or in combination) to the piglets and reported superior growth performance in the pre and post weaning periods with yeast +MOS(YMOS), followed by Yeast + lactobacillus(YL)and control (C) with higher profit. Better FCR in pigs with yeast+ lactobacillus and yeast + MOS was observed (Bera & Samanta ,2005; Siuta 2000; Tardani and Terreni 1996).

However, Charles and Duke (1978) observed no effects Incosistent resuts observed with the supplementation of probiotics were due to the following reasons.

- Variations in bacterial cultures used
- Age and species of animals
- Feed composition and feed form
- Feeding management practices adopted.
- Dose levels and Different strains of organism used
- Interaction with other dietary feed additives (Chesson 1994)

ANTIOXIDANTS: Nutrients in the body on oxidation release energy for various metabolic processes , physiological activities and to transform dietary nutrients into body tissue along with generation of heat. Auto oxidation results in the production of free radicals which damage the cellular tissue and cause many disorders (Lerson & Summer, 2002). To prevent auto oxidation antioxidants are frequently used. Nutritional antioxidants are very helpful in reducing physiological stress both at an organ and cellular level due to free radical formation.

Feed antioxidants help the birds and pigs by

- Protecting the feed nutrients during storage.
- Helping the absorption of the oxidation sensible substances in the GIT.
- Reducing aging by keeping the membrane intact.
- Enable the system for better exploitation of genetic potential

In poultry diets mostly vitamins A, beta- carotene, E, C and its calcium and sodium salts, ethoxyquin, lecithin, butylated hydroxytoulene (BHT), propyl gallate, chelated metal ions are commonly used as an antioxidants. The beneficial effects of antioxidants are due to their scavenging nature for free radicals (Bulger & Hilton, 1998), maintaing the potency of dietary vitamins and stimulating bird's immuno- responsiveness to infections. Antioxidant defence system includes the enzyme superoxide dismutase, catalase, and glutathione peroxidase. During stress free radicals in the body increase while the level of these enzymes decrease. Ascorbic acid also plays a role in collagen synthesis, carnitine synthesis along with its primary function of antioxidants (Gross et al, 2000). It scavenges neutrophill oxidants, hydroxyl radicals, hydrogen peroxide and hypochlorous acid (Bulger & Helton, 1998). Raju et al (2005) revealed that herbal Vit C (0.025%) improve the performance of bird by alleviating the effect of aflatoxicosis. Similarly the primary physiological role of Vitamin E is to act as antioxidant (Matthai, 1996). Many studies have shown that supplementation of Vitamin A, C & E can attenuate the side effects of extreme environmental stress (Kafri & Cherry, 1984, Njoku, 1986). Brahma Rasayana a polyherbal antioxidant was found useful in ameliorating the effects of free redicals generated due to heat stress (Ramnath et al, 2007). Herbs like garlic, green tea, amla also posses antioxidant properties.

ORGANIC ACIDS/ ACIDIFIERS:. Organic acids (C1-C-7) are widely distributed in nature as normal constituents of plants or animal tissues. They are also formed through microbial fermentation of carbohydrates mainly in the large intestine, and are found in sodium, potassium or calcium form.Organic acids posses both the antibacterial and anti mould activities and therefore have long been used as preservative to prevent spoilage of by checking microbial growth and are also used to maintain the proper gut health. Organic acids are very efficacious when their use is adapted to the physiology and anatomy of poultry birds

Types of Acidifiers: Generally two types of acidifiers are used in the feed industry.

1. Feed Acidifiers: These lower the pH of the feed and inhibit the growth of pathogenic microflora. This inhibition reduces the micro flora competing for the host nutrients and prevent the occurrence of diseases which results in better growth and performance.
2. Gut Acidifiers: Gut acidifiers (organic acid) acidify the intestinal tract and modulate the intestine bacterial population in a positive and natural way. Since many harmful bacterial species have pH optimum for their growth around 7 where as useful bacterial species such as *Lactobacillus* and *Enterococcus* have their best growth pH around 6. Maintenance of healthy gut for proper productivity is of utmost importance. Amongst various options available to poultry and pig feed industry, short chain fatty acids have shown tremendous promise in maintaining gut health through their varied modes of action.

Selection of Organic Acids: The antimicrobial activity of organic acids is related to reduction in pH and its ability to dissociate, which is determined by the pKa value of the respective acid and the pH of the surrounding environment. The organic acids are lipid soluble in undissociated form. More as undissociated form of organic acids, increases the efficacy. Hence the selection of organic acid is very critical in determining the efficacy of the product. Table 8 depicts physiochemical properties of few SCFA and their salts

Functions of Acidifiers: Acidifiers have various functions in monogastric animals.

- Help in maintaining an optimum pH in stomach
- Stimulate feed consumption
- Improve growth rates
- Improve feed conversion ratio
- Inhibit the growth and colonization of pathogenic bacteria
- Prevents damage to epithelial cells of intestines
- Reduce microbial competition with host for nutrients
- Reduce endogenous nitrogen losses
- Lower the incidence of sub clinical infections
- Reduce the production of ammonia and other growth depressing microbial metabolites
- Increase pancreatic secretions
- Increase protein and amino acid digestibility by correcting activation and function of proteolytic enzymes
- Improve energy digestibility
- Increase mineral digestibility as acid ion complex with minerals
- Serve as substrates in intermediary metabolism and have energy content
- Check problem of Salmonella, E. coli, entritis and diarrhoea in pigs
- Check entritis and mortality syndrome.

Supplementation of organic acids improve the weight gain, feed consumption and feed utilization (Denli et al, 2003) reducing the production of toxic components by pathogenic bacteria and reduces the colonization of pathogens on the intestinal wall, thus preventing the damage of the epithelial cells (Langhout,2000) .In poultry diets organic acids are mainly used in order to sanitize the feed to avoid the problems releated with salmonella (Berchieri & Barrow,1996). However Waldroup et al (1995) reported the inability of citric acid at the dietary concentration up to 1 % to prevent the salmonella colonization in caeca. In poultry nutrition organic acid have not gained as much attention as in swine nutrition (Langhout, 2000). Edwin (2000) reported that addition of 2% lactic acid to the diet without growth promoters increased the weight gain by 2.6 % with improved FCR.

Propionic acid based products were found effective in alleviating the enteritis and mortality syndrome in turkey poults (Roy et al 2002). Several organic acids like citric acid, fumaric acid, formic acid, propionic acid were tried on pig for their impact on the growth performance (Partanen & Mroz, 1999). Their supplementation in weaning pig diets give most pronounced impact on the growth performance (Roth & Kirchgessner, 1998). Blank et al (1999) reported that the incorporation of organic acids into nursery pig rations has been shown to reduce bacterial load and increase the digestibility of energy and amino acid in the ileum, resulting in improvement in feed efficiency and reduction in the incidence of diarrhea. These pigs often suffer from digestive problems due to infection of *E coli.* An insufficient production of HCl, digestive enzymes and feeding of high protein pre starter diets are another reasons for the digestive upset at this stage. Supplementation of organic acid increases the gastric proteolysis, protein and amino acid digestibility. The acid anion has been shown to complex with Ca, P, Mg, and Zn which results in an improved digestibility of these minerals. Kirchgessner and Roth (1988) also revealed the role of organic acid as substrates in the intermediary metabolism. Supplementation of 1.5% citric acid to control diets did not significantly effect the pH, concentration of VFA's / non VFA or microflora (total anarobes, *Lactobacilli, Clostridia*, *E. coli*) in the contents from the stomach, jejunum, caecum or lower colon of weanling pigs (Risley et al, 1992,). Similar results were reported for the Fumaric acid (Risley et al, 1992). Sodium fumarate when added to a control pig diet at a level of 0.3%, no significant effect on the concentration of SCFA and the density of lactobacilli or E coli along the GI tract was observed (Sutton et al, 1991). Supplementation of 1 % lactic acid lowers the gastric pH (Thomlinson & Lawrence, 1981) and reduced the level of *E coli* in the duodenum and jeunum of 8-week-old piglets (Cole et al 1968). Addition of formic acid or potassium diformate reduces the pH, (Fevrier et al 2001) and number of coliform bacteria in stomach, duodenum, jejunum and rectum of growing pigs (Overland et al, 2000). Waldroup *et al* (1995) reported the reduction of caecal pH with the addition of a formic acid/ propionic acid blend in a concentration of 1% in the broiler chicken. Supplementation of benzoic acid though not approved as an additive or preservative in the pig or poultry feed but it is extensively used as food preservative in human nutrition. The preliminary results from the experiment with broiler chicken indicate the positive influence on growth (Engberg 2001). It seems that these short chain fatty acids can nearly compensate for the effects of antibiotic growth promoters in pigs, although these effects are less consistent (Partanen and Mroz, 1999).

Feeding of Excess of Organic acids: Excess level of strong dietary acid can reduce the pH too quickly after feed ingestion but the stomach may not develop its parietal secretary cells that produced HCl. This inhibits the normal gut development. Therefore use of organic acids must be done judiciously.

Mode of action: Over the years, it was thought that a pH reduction of the gastrointestinal tract (GIT content was the mode of action. But new research has proven differently. Research in the food preservation field has brought clear explanations on the mode of action of organic acids on bacteria and numerous trials have shown that the concept works both in pigs and poultry. More likely, the organic acids in poultry might play a direct role on the GIT bacteria population, reducing the level of some pathogenic bacteria (*C. perfringens*) and mainly controlling the population of certain types of bacteria that compete with the birds for nutrients. Animal has the capacity of to maintain its GIT environment homeostasis in order to warrant the normal functioning of all digestive functions. Also, the strong buffering capacity of the feed prevents any significant GIT pH modification. Logically, organic acids added to feeds should be protected to avoid their

dissociation in the crop and in the intestine (high pH segments) and reach far into the GIT, where the bulk of the bacterial population is located.

State of the organic acids whether undissociated or dissociated is extremely important to define their capacity to inhibit the growth of bacteria. As a general rule, more than ten to twenty times the level of dissociated acids to reach the same inhibition of bacteria are required as compared to undissociated acids. The antibacterial capacity of organic acids is, at a low pH. At a pH below 3.0-3.5, almost all organic acids are very efficacious in controlling bacteria growth.

The mode of action of organic acids on bacteria is as under:

- Undissociated organic acids entering the bacterial cell.
- Bacteria membrane disruption (leakage, transport mechanisms).
- Inhibition of essential metabolic reactions (ex. glycolysis).
- Stress on intracellular pH homeostasis.
- Accumulation of toxic anions.
- Energy stress response to restore homeostasis.
- Chelation as permeabilizing agent of outer membrane and zinc binding.

The key basic principle on the mode of action of organic acids on bacteria is that non-dissociated (non-ionized) organic acids can penetrate the cell wall and disrupt the normal physiology of certain bacteria E. coli, Salmonella spp., C. perfringens, Listeria monocytogenes, Campylobacter spp. This is called "pH-sensitive," meaning that they cannot tolerate a wide internal and external pH gradient.

The dual mode of action of organic acids make them more attractive as at low pH they do not disassociate where as at higher pH, the acid disassociates .The undissociated form oforganic acid prevents the cell emembranes.In the alkaline environment of bacterial cytoplasm, the organic acids disassociate and release H+ ions and acidifies the cell contents., Microbial enzyme working is inhibited due to low pH in the cell and the distressed cell start pumping out the H+ ion in order to return to equilibrium. The energy required to eject the H+ ion is thought to weaken the already distressed cell, there by slowing growth and reproduction. Anions of organic acids deactivate RNA transferase enzyme, which damage the nucleic acid multiplication process and eventually result in death of the organism.Â

Pumped out H+ ion acidify the intestinal contents which favours the acid producing bacteria (Lactobacilli, bifidobacteria) and inhibits the acid hating bacteria (Salmonella, E Coli, Campylobacter sp).
All acids have different dissociation constant (pKa values), low pKa values indicates strong acidification power. Some acids have multiple pKa value and these acids can dissociate at different parts of the GI tract. Acids can be used singly or in combinations. By combining several organic acids, which disassociate at different pH levels, it is possible to exploit the effects of both modes of action over a larger proportion of the range of pH found in the intestines.

Problems of feeding Organic acids: The unprotected acids can cause a fast drop in pH and by dissociating quickly in the stomach or upper small intestine may not be effective. Without protection, organic acids are readily dissociated in the first part of the gastrointestinal tract and rendered useless. Acids can also be used in protected form by using the encapsulation technology for their slow release in the GI tract

ESSENTIAL OILS: Essential oils are highly concentrated extracts produced by refinement of botanicals by hydro-distillation. Essential oils are standardised products, often based on a blend of plant metabolites such as allylisothiocyanates, thymol, carvacrol, capsaicin, cinnamaldehyde, piperin etc. Any class of volatile oils obtained from plants, possessing the odor and other characteristic properties of the plant are used chiefly in the manufacture of perfumes, flavors and pharmaceuticals (extracts after hydro distillation).

Plants contain hundreds of substances with different properties but essential oils are composed mainly of nine groups (and many sub-groups) of molecules that are of interest to us. There are many chemical constituents but no two oils are alike in their structure and effect. One must distinguish between non-purified plant extracts containing numerous different molecules interacting and pure active compounds, either extracted from plants or synthesized (nature identical). According to the plant chosen, one or more active compounds are dominant and the quantity found will differ according to factors like plant variety, soil, moisture, climate, time of harvest and other factors

Functions of Essential Oils: Nutritionally, metabolically and toxicologically, we have a clear interest in using as low levels of essential oils as possible in animal nutrition. Essential oils are extremely potent substances, which are rapidly absorbed in the duodenum and can not interact with the microflora

Essential oils are used as

1. Flavouring agents to increase their attractiveness of the feeds.
2. To reduce feed intake
3. Manipulate/disturb GIT microflora
4. Antimicrobial.
5. Antioxidant.
6. Coccidiostat.
7. Antiviral properties.
8. To increase the digestive enzyme secretions.
9. Increase the appetite
10. Improve the immune function.
11. Improve the performance and
12. Accumulation in animal tissues and products

There are reports of synergy between organic acids and essential oils. The synergy is thought to come from the ability of the essential oils to weaken bacterial cell walls, increasing its permeability to the organic acids.

Mode of Action: It is extremely difficult to generalize on the mode of action of essential oils (EO) on bacteria and yeasts because each has different properties and each type of microorganism has a different sensitivity. Generally, Gram+ bacteria are considered more sensitive to essential oils than Gram- bacteria because of their less complex membrane structure.

Essential oils work by inhibiting the growth of the bacteria. These compounds influence the biological membranes of bacteria. The cytoplasmic membrane of bacteria has two principal functions i.e. 1. barrier and energy transduction, which allow the membrane to

form ion gradients that can be used to drive various processes and 2. formation of a matrix for membrane-embedded proteins influencing the ATP-synthase complex, sharply reducing intracellular ATP pool through a reduction of ATP synthesis and increased hydrolysis; education of the membrane potential, which is the driving force for ATP synthesis; the membrane becoming more permeable to protons; and reduction of the internal pH.

Judicious use of Essential oils: Most essential oils are generally recognized as safe (GRAS) but they must be used properly and cautiously because they can be toxic (allergens), potent sensitizers and their odor and/or taste may contribute to feed refusal. They are also very volatile and will evaporate rapidly, leading to large variation in concentration in the finished products.

Essential oils are very rapidly absorbed in the duodenum and cannot interact with the microflora.

ENZYMES: Non starch polysaccharides or NSP (cellulose, glucans and xylans etc)) of the cereal grains (Henry, 1985) like wheat, rye, oats possess antinutritive activity (Annison & Choct, 1991) which leads to the formation of viscous gel in the gut that intrferes the proper absorption of nutrients (Choct & Annion, 1992) and also produces sticky droppings in poultry. Similarly phytic acid and its salts as phytates present in the feedstuffs also binds minerals, carbohydrates, proteins and form insoluble complexes which make these nutrients especially minerals like phosphorus unavaiable to the monogastrics and are excreted in faeces. The supplementation of exogenous enzymes in the diets decrease gut viscosity and improve the availability of nutrients from feed, lower the feed cost and help in reducing the environmental pollution by minimizing the waste excretion. Exogenous enzymes in the diets of young animal complement the endogenous enzymes. Their use in the poultry and pig feed industry has become a routine (Bedford, 1997, Sikka, 2003.

The enzymes in pig and poultry feeds are added to achieve the following goals

- To supplement the endogenous enzymes especially to young birds and pigs due to immature digestive system
- To counter ANF's present in feed
- To increase the availability of dietary nutrients.
- To improve the AME level of the feeds
- To release the bound nutrients
- Pre treatment of certain feeds / ingredients such as feathers and offals.

Phytase enzyme was found to improve the availability of phyatate phosphorus as well as other organic nutrients (Sebestian et al, 1998, Singh & Sikka 2006). Eeckhout et al (1992) revealed that supplementation of phytase at 1000U/kg diet increases P digestibility by 36-55% in maize soybean and 54-68% in wheat soybean diets given to 5 week old weaner. The supplementation of phytase improve performance and mineral retention (Yi et al 1996, Singh & Sikka, 2006)

Similarly supplementation glycosidase has been found to increase the energy utilization in birds. Higher body weight gain and better feed efficiency in Japanese Quails with supplementing of 0.05%, non starch polysaccharidase (Edwin et al, 2004) and in broiler (Srivastava et al, 2005) with enzymes mixture of amylase, cellulose, lipase and protease and in weaner pigs (Owsley et al 1986) with diminsihing digestive disturbance (Kitchen 1997, Partridge & Hazzledine,1997). The improvement was more in young pigs than

older one (Bohme et al 1990).The use of beta glucanase and xylanase are beneficial with high fiber grains like wheat, barley and their by products (Chesson, 1987, Sikka & Chawla 2002).Thomke et al (1980) also reported that b-glucanases could improve performance in barley fed pigs. Alpha galactosiadse is used to breakdown the galactose units in raffinose and stachyose found in soyabean. The efficacy of enzyme supplementation depends upon types of diet, animals, chemical linkage in the substrate that need to be cleaved etc.

AUGMENTATION OF IMMUNITY- IMMUNO MODULATORS: Nutrition and disease have close connection as the nutritional status of animal influence immunological function and resistance to disease. Health status of the organism is influencing the animal's nutritional requirements. Many nutrients like protein & energy (Praharaj et al 1999), methionine (Tsiagbe et al 1987, Swain & Johri 2000), Vitamin A (Friedman & Sklan 1997), Vitamin E &Se (Swan et al 2000, Areceli & Saavedra, 2003, Hooda et al 2005, Singh et al 2006), Vitamin C (Amakya anim et al 2005) and trace element like Zn, Fe, Cu & Mn (Dardenne 2002) have the immuno modulating ability. In pigs nucleotides (Tibbetts 2000), ß glucans (Diluzio & Jacques 1985), vitamins, PUFA, antibodies from products such as blood derivatives (eg, plasma protein), freeze dried eggs containing pig related antibodies and possibly some whey protein products have been reported to improve the immune response. Reduced immune activity promotes growth by increasing appetite and partitioning nutrients to growth.

IDEA CONCEPT: Immuno modulation through nutrition gave birth to a new concept the '**IDEA', which** stands for Impulse, Digestibility, Economics and Advance. The IDEA concept seeks to

- Enhance immunity development
- Giving opportunity for better nutritional management
- Reduce feed costs
- Reduce intestinal challenges by coccidia and bacteria without the use of drugs
- Conditioning the gut for better coccidiosis management especially in broilers.

The IDEA concept is simple but an innovative approach to feed management which redefines the birds nutritional and management needs during critical phases.

Supportive Dietary Modification: Bacteriostatic approach is supported by alteration in the diet to reduce the amount of substrate available to the intestinal microflora. Diets must be modified to reduce "By-Pass Nutrients"! This is best achieved through increased digestibility of ingredients by the addition of enzymes, herbs, probiotics, acidifiers etc. The aim is to reduce the protein and carbohydrate fraction of the diet, which can escape digestion and absorption and remain available as a nutrient source for microbial fermentation by intestinal microflora. Bacterial fermentation of indigestible protein produces ammonia and biogenic amines, which are toxic and increase the risk of diarrhoea. Piglet starter diets must be highly digestible and must encourage a shift to protein fermentation in the hindgut by being 'carbohydrate' deficient. The addition of fermentable carbohydrates (prebiotics) to pig diets reduces protein fermentation through increased carbohydrate fermentation in the hindgut. The reduced efficiency of bile salts can be countered by adding emulsifying agents (lecithin) directly to the diet and by improving the saturated to unsaturated fatty acid ratio in the diet to aid absorption. Alterations such as switching from DL-Methionine to Liquid MHA-FA, an organic acid, is another small change which can increase the antimicrobial status of a pig diet. The

efficiency of the intestinal epithelium structure and function can be upgraded with the use of Betaine

Future research needs:

1. Efforts be made to identify the specific herbs for specific purposes and the mechanism how they improve the performance.
2. It is the need of the hour to identify the oligosaccharides and other compounds present in the feedstuffs that promote the development of beneficial microbes in the gut.
3. Though a lot of work on the use of enzymes in pig and poultry diets has been done but the area of ingredient specific enzymes, their doses, interactions need to be explored.
4. Other alternates to the antibiotics involves the feeding of specific antibodies to neutralize pathogenic organism. The immune protein are sensitive to heat treatment of processing. So more focus is needed on it.
5. Nutritional strategies should be developed for the early and maximum development of villi morphology for enhanced digestion and absorption.

Summary

To day we now have the crude products which will efficiently bridge the gap between AGP's and a new generation of products being developed with the aid of molecular biology. The challenge for pig and poultry producers is to find suitable, reliable and cost effective management routines and feed additives for a sustainable and successful production. Health and hygiene will be key to success without AGP's. There could not be a better time to agree or review a health plan for each pig and poultry unit. Cleaning and disinfection routines should also be reviewed and upgraded. The quality of stockmanship and management is also fundamentally important in tackling post weaning growth performance and post weaning diarrhea in pigs. The removal of AGP's from pigs and poultry diets will no doubt increase the cost of production AGP's are thought to have altered the intestinal bacterial population in a way that yielded benefits from intestine-bacteria interactions, which resulted in improved performance of poultry birds and pigs. A failure to replace AGP's will result in an increase in adverse intestine-bacteria interactions. Bacteria are important in the regulation of the intestinal function and immunity. Bacteria have a direct influence of the genetic programming of the intestines through direct association with the intestinal cell surfaces. Bacteria communicate with mucosal epithelial cells and modulate cytokine profiles. The expansion and function of the immune system necessitates exposure to bacterial antigens. Dietary additives, nutritional modifications and improved farm hygiene and management can prevent performance and economic loss .

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Table7: Herbs used in poultry feeds

Herb	Tentative claim	Refernce
Garlic (Allium sativum)	Wonder drug in herbal world Antibacterial, antifungal, anti-inflammatory, antioxidant, Improve immunity, Helps the birds to get rid of internal parasites. Helps in removing the lead from bird muscle Reduce level of cholesterol in plasma, liver, breast & thigh muscle without effecting growth and feed efficiency.	Hanafy et al (1994) Naganawa et al 1996 Kopnjufca et al 197
Haldi or Turmeric (Circuma longa)	Immuno stimulater Hepato stimulator Improve feed intake and performance	Wenk & Messikommer, 2002 Samarasinghe & Wenk,2002
Amla (Phyllanthus emblica)	Immuno stimulater Hepato stimulator	
Kuar gandal (Aloe Vera)	Improve the gastrointestinal condition Improve resistance to Infectious Bursal disease & New Castle Disease Immunostimulant	
Ginger (Zinziber officinale)	Antifungal, Effective against diarrhea related with cold weather	
Bitter gourd (Memordica charantia)	Poultry worms, Colitis, Blood containg diarrhea	Lans & Brown 1998
Basil (Ocimum sanctum)	Antiviral effect Immunomodulater	Mathew et al, 2001
Neem (Azadirachta indica)	Antimicrobial Anticoccidial	
Thyme (Thymus vulgaris)	Excellent livertonic, Enhance detoxification by inducing liver enzymes	Teran Somaza et al, 1982
Astragallus	Antiviral effect, Effective against Marecks disease, Immunostimulant	Kumar & Smith, 2000
Blue green algae (Spirulina platensis)	Improve macrophagic function Increase antibody production	Querashi et al,1995,
Yucca extract (Yucca sahidigra)	Reduction in ascitis mortality	Menocal (1995) Headon et al 1991
Ashwaganda (Withania	Improve appetite weight gain	Vaidya & Kulkarni, 1991;

somnifera)	Adaptogenic Antistress	Singh et al 2003
Jiwanti (Holostimma adakoien)	Improve body weight gain Feed conversion efficiency	Ritu 2004
Bhringraj (Eclipta alba)	Improve body weight gain Improve FCR Hepato protective	Ritu 2004

Table8. Some physicochemical properties of most common SCFA and salts

Sr. No.	Name of acid	Physical form	Mol.wt	GE (MJ/kg)	constant (pKa)	CR2	Odour
1.	Formic	Liquid	46.03	5.7	3.75	++(+)	Pungent
2	Acetic	Liquid	60.05	14.6	4.76	+++	Pungent
3.	Propionic	Oily liquid	74.08	20.6	4.88	++	Pungent
4.	Butyric	Oily liquid	88.12	24.8	4.82	+	Rancid
5.	Lactic	Liquid	90.08	15.1	3.86	(+)	Sour milk
6	Sorbic	Solid	112.1	27.85	4.76	(+)	Mildly acrid
7.	Fumaric	Solid	116.1	11.5	3.02/4.38	0 to (+)	Odourless
8	Malic	Solid/Liquid	134.1/	10.0	3.46/5.10	(+)	Apple
9.	Citric	Solid	192.1/	10.2	3.1/4.8/6.4	0 to ++	Odourless
10.	Ca-formate	Solid	130.1	11.0		0	Neutral
11.	Ca-lactate	Solid	308.3/	30.0		0	Neutral
12.	Ca-propionate	Solid	184.1	40.0		0	Neutral
13	.K-diformate	Solid	130.0	11.4		0	Neutral
14.	Ca-butyrate	Solid	214.0	48.0		0	Rancid
15	.Mg-citrate	Solid	214.4/	10.0		0	Neutral
16.	Na-lactate	Solid	112.1	15.0		0	Neutral

Table9: Exogenous enzymes frequently used in poultry feeds.

Nutrient	Enzyme
Carbohydrates	Amylase, Arabinase, Cellulase, Glunase, Hemicellulase, Pectinase,Xylanase
Protiens	Acid protease and Alkali Protease
Fats	Lipases, Esterases
Others	Phytase and Tannase

Table 10 IDEA CONCEPT

IDEA	Days	Main Focus	FEED	Impact
Impulse	0-14	• Intestinal and immune system • Maximum duodenum villi development.	Critically review and evaluate protein, energy, vitamins and trace mineral. Newly hatched chicks need immediate access to feed and water to set the stage for good performance later	Establishment of intestinal microflora, Maximum efficiency of bones and muscles formation .Determines the number of enterocytes.
Digestibility	15-28	Minimum intestinal irritation minimize invasion of secondary bacteria. Prevent intestinal disruption	Replace abrasive or less digestible ingredients with high quality, highly digestible feeds with an optimal enzyme dosage. Adjust protein levels as needed. Avoid dietary changes to prevent intestinal disruption.	Least intestinal irritation..More digestibility. Less growth of undesirable bacteria
Economic	>30	Maximum daily growth Maximum feed efficiency.	Provide recommendation concenetration of nutrient	Establishment immunity. Higher daily gains and Feed consumption.
Advance		An advancement in the traditional thinking with respect to manipulation of immunization, management ,nutrition.	Provide recommendation concenetration of nutrient	Improvement in overall performance